

Antimicrobial resistance and food safety: A public health challenge



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CDC estimates...

1 in 5 resistant infections are caused by microorganisms from food and animals

IMPACT Some resistant infections cause...

mild illness

severe illness and may lead to death

About **1 in 5** resistant infections are caused by germs from food and animals.

Source: *Antibiotic Resistant Threats in the United States, 2013*

Learn more about antibiotic resistance and food safety at www.cdc.gov/foodsafety/antibiotic-resistance.html
Learn more about protecting you and your family from resistant infections at www.cdc.gov/drugresistance/protecting_yourself_family.html

CDC

CS200910

EXPOSURE People can get sick with resistant infections from...

contaminated food

contaminated environment

...through contaminated food or contaminated environment.

Is "resistance"
always used
correctly in
the context of
AMR?

Antimicrobials
Tolerance
Resistance
Sanitizers
Disinfectants
Antibiotics

OUTLINE

- Scope of the problem
 - Resistance, tolerance and persistence
- What happens in food processing environments?
- AMR, sanitation & co-selection

Let's clarify some terms...



Antimicrobials

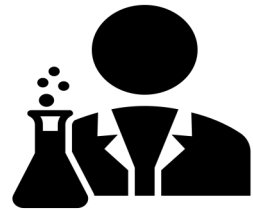
Collective term for sanitizers, disinfectants, antibiotics



Biocides (defined in Europe)

Products intended to destroy, render harmless, prevent the action of, or otherwise exert a controlling effect on any harmful organism by chemical or biological means

Let's clarify some terms...*continued*



Sanitizers

A substance, or mixture of substances, that reduces the bacteria population in the inanimate environment by significant numbers but does not destroy or eliminate all bacteria.

US EPA:

- 5 logs (99.999%) for food contact surfaces;
- 3 logs (99.9%) over 5 min for non-food contact surfaces.



Disinfectants

A substance or mixture of substances that destroys or irreversibly inactivates bacteria, fungi, and viruses but not necessarily bacterial spores, in the inanimate environment.

US EPA:

- 6 logs (99.9999%) for both food contact and non-food contact surfaces;
- Virus control determined by product approval.

Resistance

- Associated with numerous molecular mechanisms
- Quantified by the **minimum inhibitory concentration (MIC)**
 - Minimum concentration of an antibiotic that is required to prevent net growth of the culture
- Measured by exposing bacteria to increasing concentrations of the antimicrobial in a standardized growth medium
- In practice, minimum concentration at which growth is not detected, usually after 16–20 hours of exposure to the antimicrobial

Tolerance

Persistence

Susceptible vs. resistant bacterial strains

Brauner et al., 2016. *Nature Rev. Microbiol.* 14:320–330.

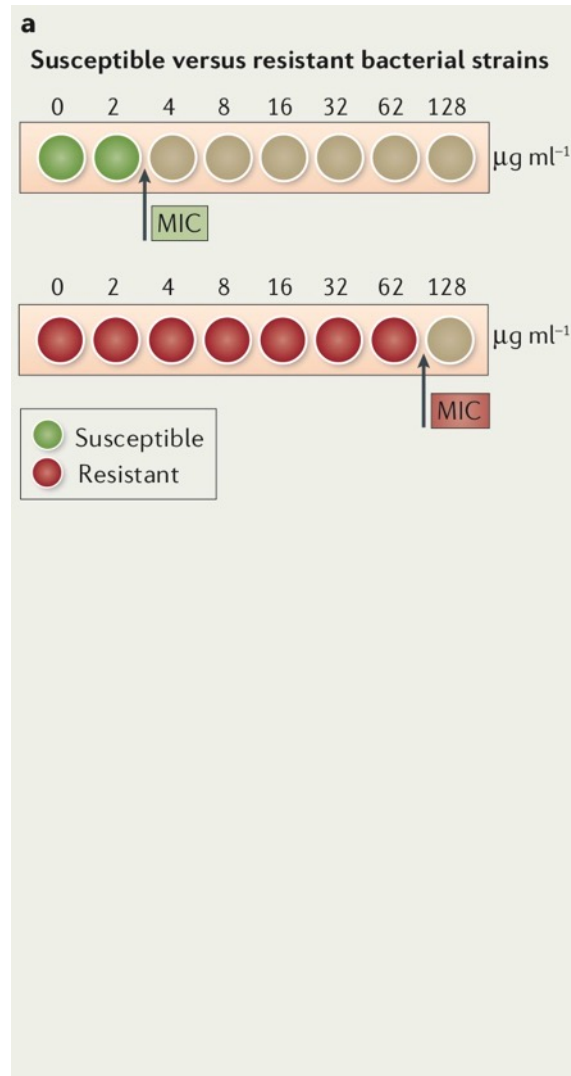


Fig. 1a | The minimum inhibitory concentration (MIC) for a strain of bacteria that is resistant to an antibiotic is substantially higher than the MIC for a susceptible strain. Colored wells represent bacterial growth, whereas wells in which the antibiotic concentration is high enough to kill the bacteria are in light brown.

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Tolerance

- The ability of a bacterial population to survive a transient exposure to antimicrobials, even at concentrations that far exceed the MIC
 - Tolerance applies only to **bactericidal antimicrobials** and not to **bacteriostatic antimicrobials**
- Tolerant strain can have the same MIC as non-tolerant strain
- Longer exposure to antimicrobial rather than a higher concentration may be required to produce the same level of killing
- Minimum duration for killing (MDK) suggested to better measure tolerance to antimicrobial exposure

Persistence

How does tolerance work?

Brauner et al., 2016. *Nature Rev. Microbiol.* 14:320–330.

- Tolerance by **slow growth**
 - Inherited...when a bacterial species or strain has an inherently slow growth rate
 - *Mycobacterium tuberculosis*
 - Non-inherited...when conditions for growth are poor
 - Triggered by external stress factors
- Tolerance by **lag**
 - Lag phase: time it takes for non-growing bacteria (e.g., under starvation conditions) to resume exponential growth when adjusting to favorable environment (e.g., when starved bacterial cells are transferred into fresh growth medium)
 - Transient phenotype that disappears when bacteria are adjusted to the new conditions

Susceptible vs. tolerant bacterial strains

Brauner et al., 2016. *Nature Rev. Microbiol.* 14:320–330.

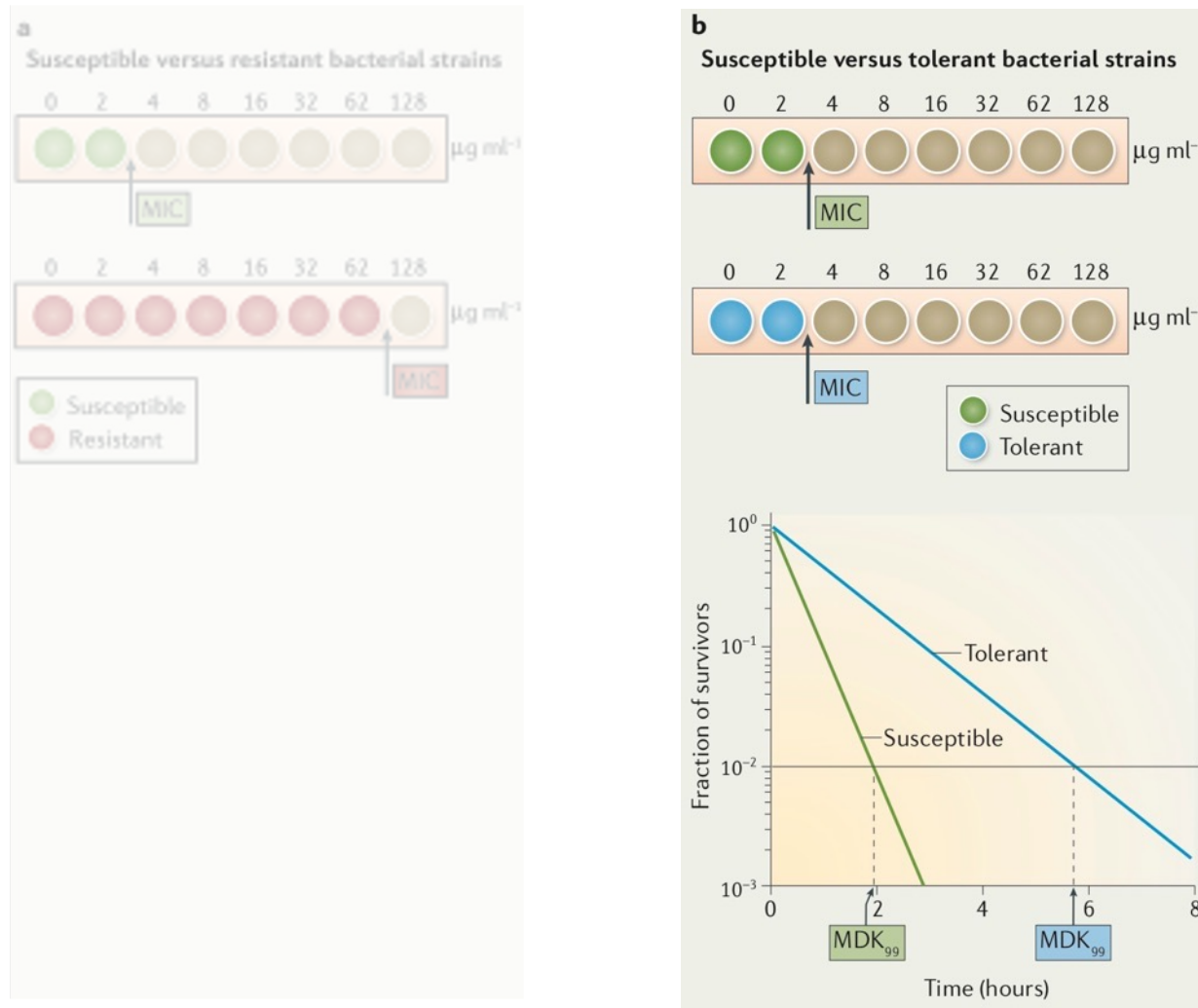


Fig. 1b | The MIC for a tolerant strain of bacteria is similar to that of a susceptible strain; however, the minimum duration for killing (MDK; for example for 99% of bacterial cell population (MDK₉₉)) for a tolerant strain is substantially higher than the MDK₉₉ for a susceptible strain

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Persistence

- Subpopulations that are not killed by antimicrobials can emerge – persisters
- Persisters are typically less than 1% of the population and are killed at a slower rate than the susceptible cells

Persistence

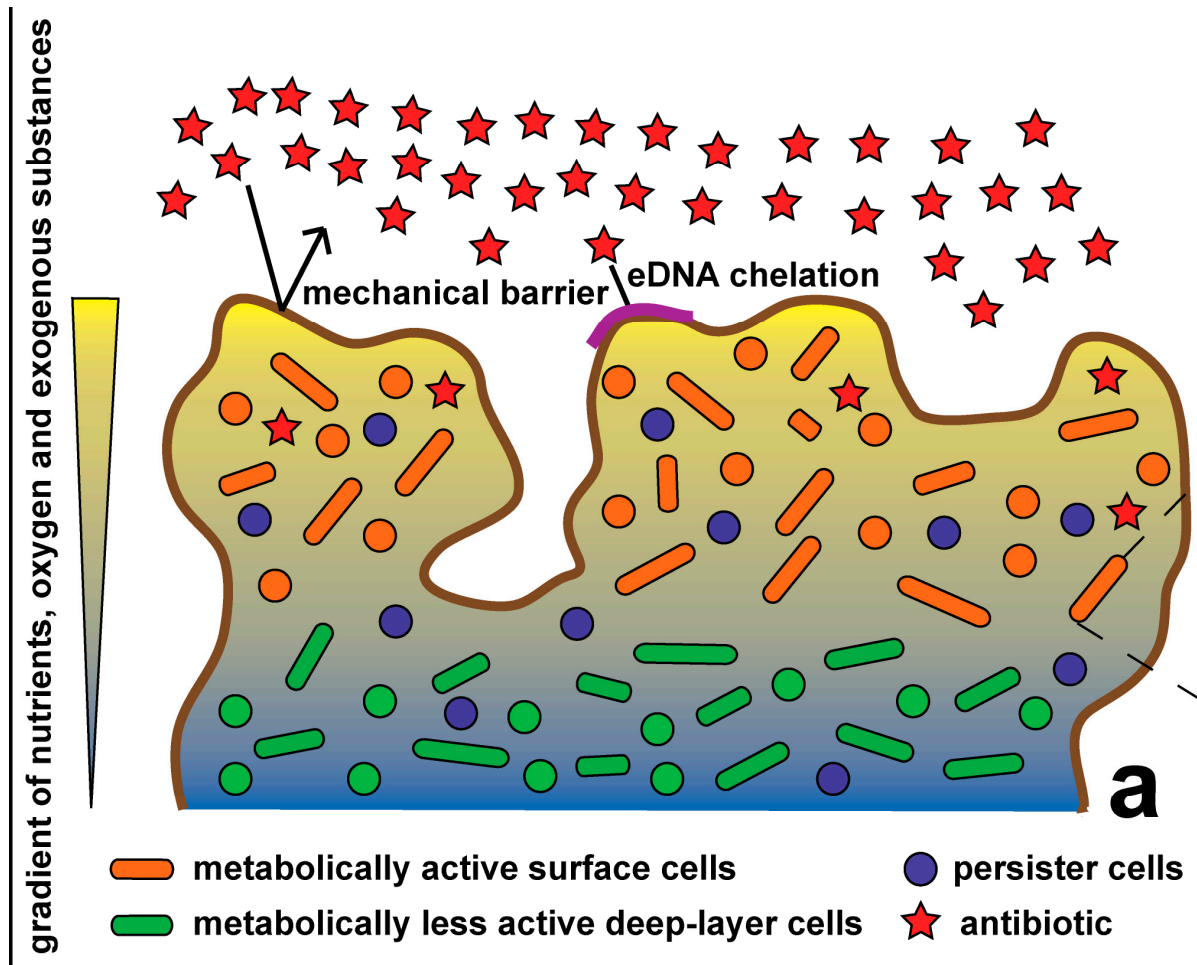


Fig 2. Antibiotic resistance at the community level. From Penesyan et al. 2015. *Molecules* 20:5286-5298.

Susceptible vs. persistent bacterial strains

Brauner et al., 2016. Nature Rev. Microbiol. 14:320–330.

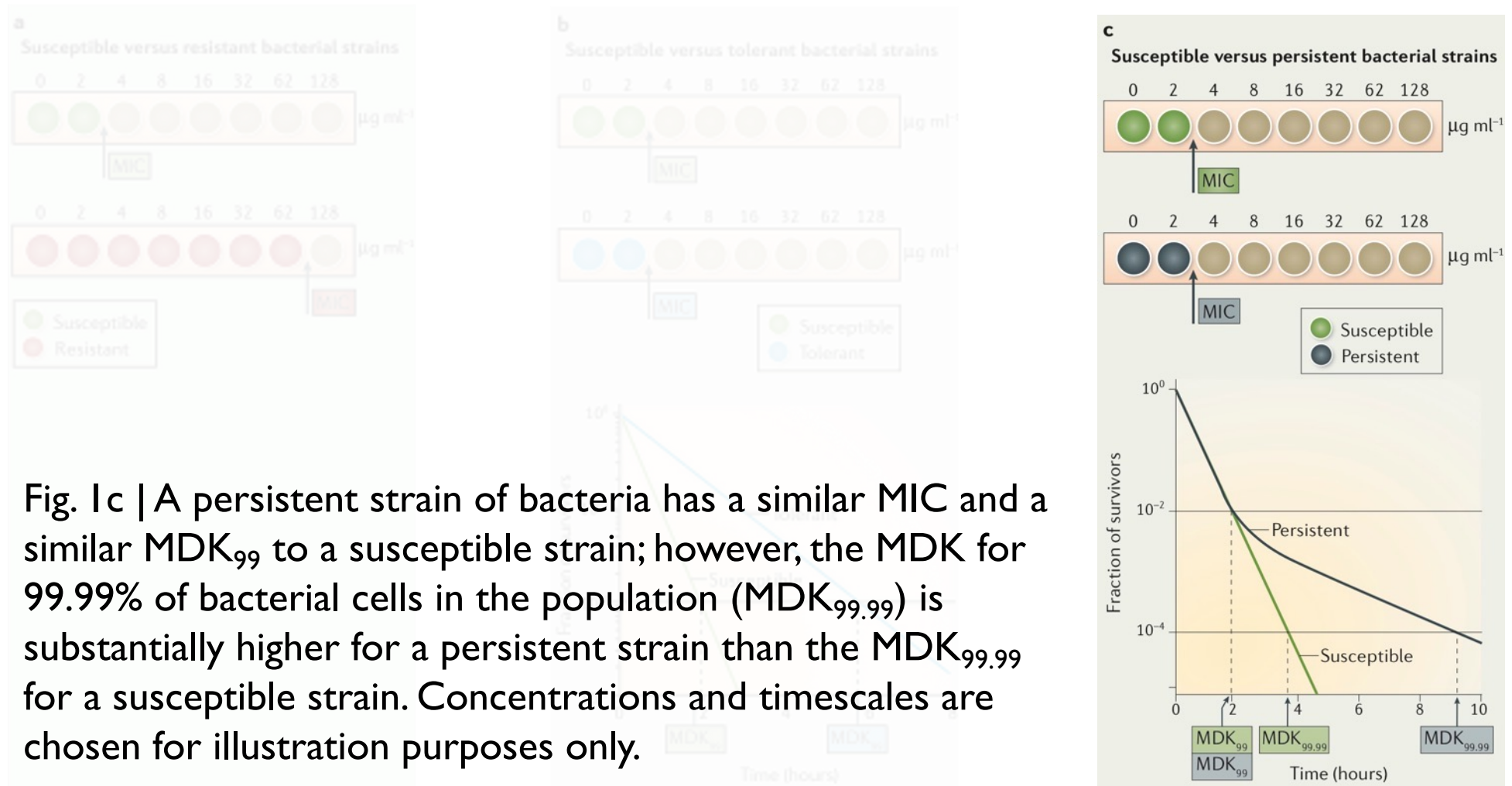
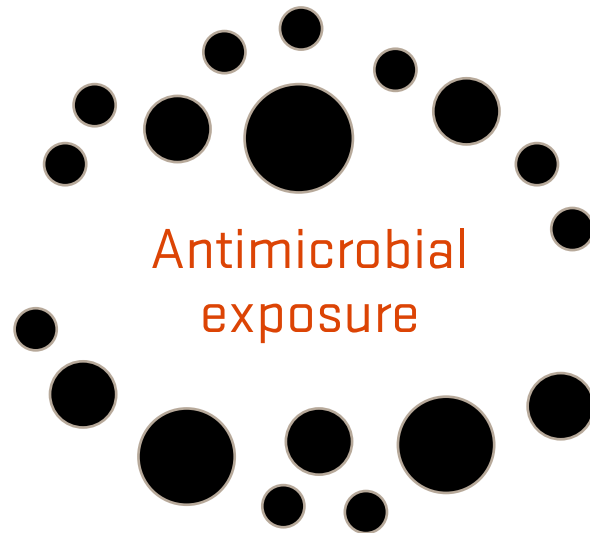


Fig. 1c | A persistent strain of bacteria has a similar MIC and a similar MDK₉₉ to a susceptible strain; however, the MDK for 99.99% of bacterial cells in the population (MDK_{99.99}) is substantially higher for a persistent strain than the MDK_{99.99} for a susceptible strain. Concentrations and timescales are chosen for illustration purposes only.



What does AMR
look like in the
food chain?

AMR in the food chain



Bacteria can be challenged with antibiotics, preservatives, heavy metals, sanitizers or disinfectants



Activation of stress responses

Leads to activation of complex bacterial stress responses

Commonly triggering the over-expression of efflux pumps, responsible for expelling the antimicrobials from the cell



Sanitizer-induced cross-resistance to antibiotics

Co-selection phenomenon

Survival advantage; persistence

AMR and food chain

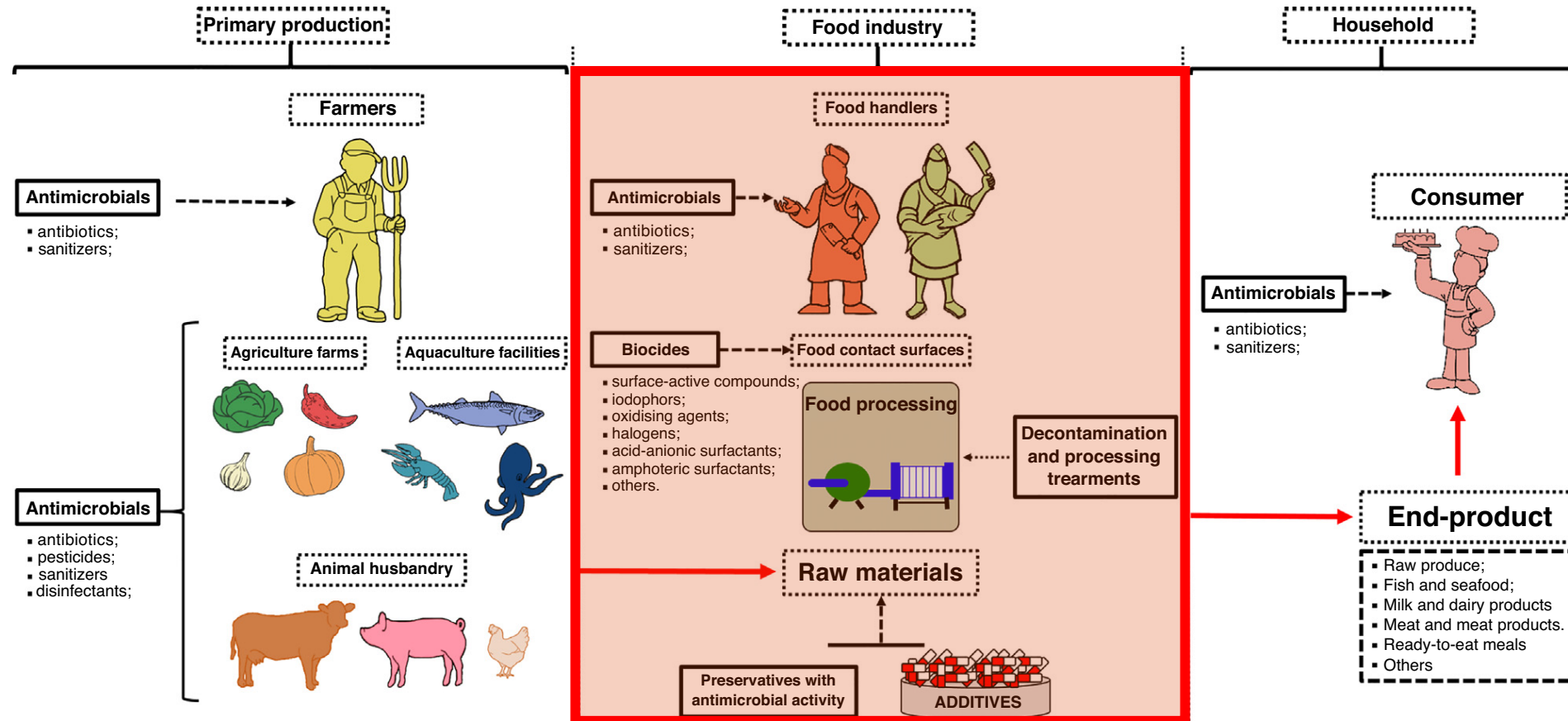
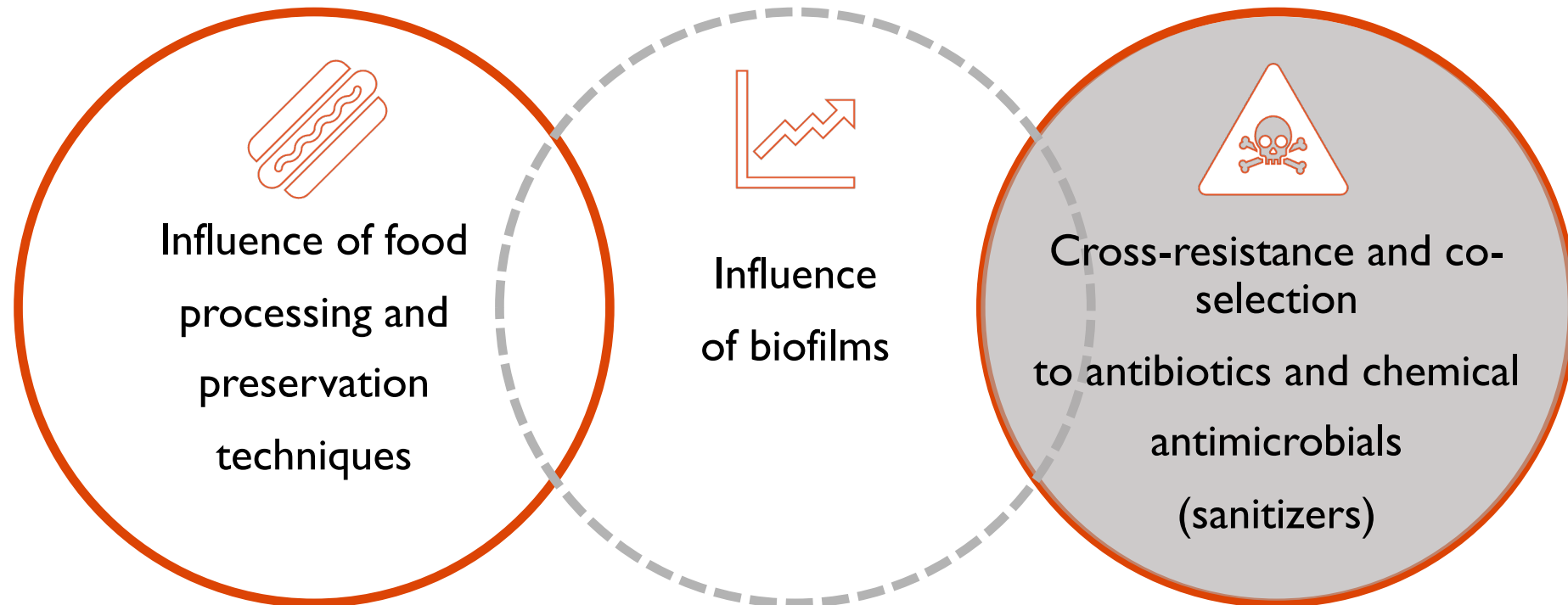


Fig. 3. Schematic overview of the main sources of antimicrobials and routes of transmission of antimicrobial resistance along the food chain. From Oniciuc et al., 2019. Current Opinion Food Sci, 30:21-26.



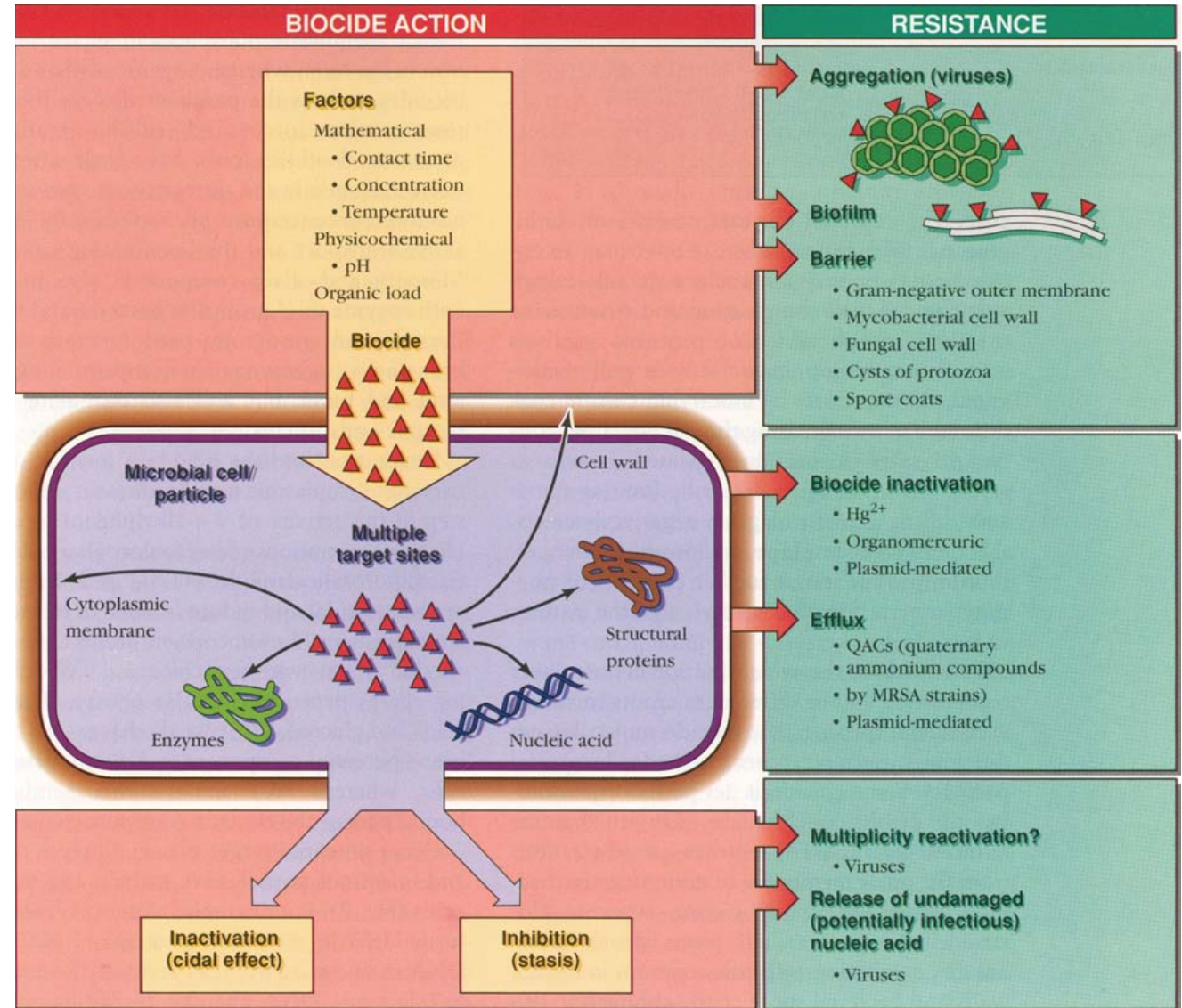
What happens in the food processing environment?

AMR and food processing environment



Antimicrobials become less effective when there is...

- Impaired uptake
- Modification or overproduction of the target sites of antimicrobials
- Absence of enzymes or metabolic pathways
- Efflux of the antimicrobial

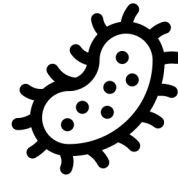


Stress-adaptation

Exposure to sub-lethal concentrations leading to intrinsic resistance and decreased susceptibility to the inducing agent and other, unrelated antimicrobials.

Cross-resistance

Resistance to antimicrobials with same molecular targets.



Consequences of stress exposures?

Co-selection

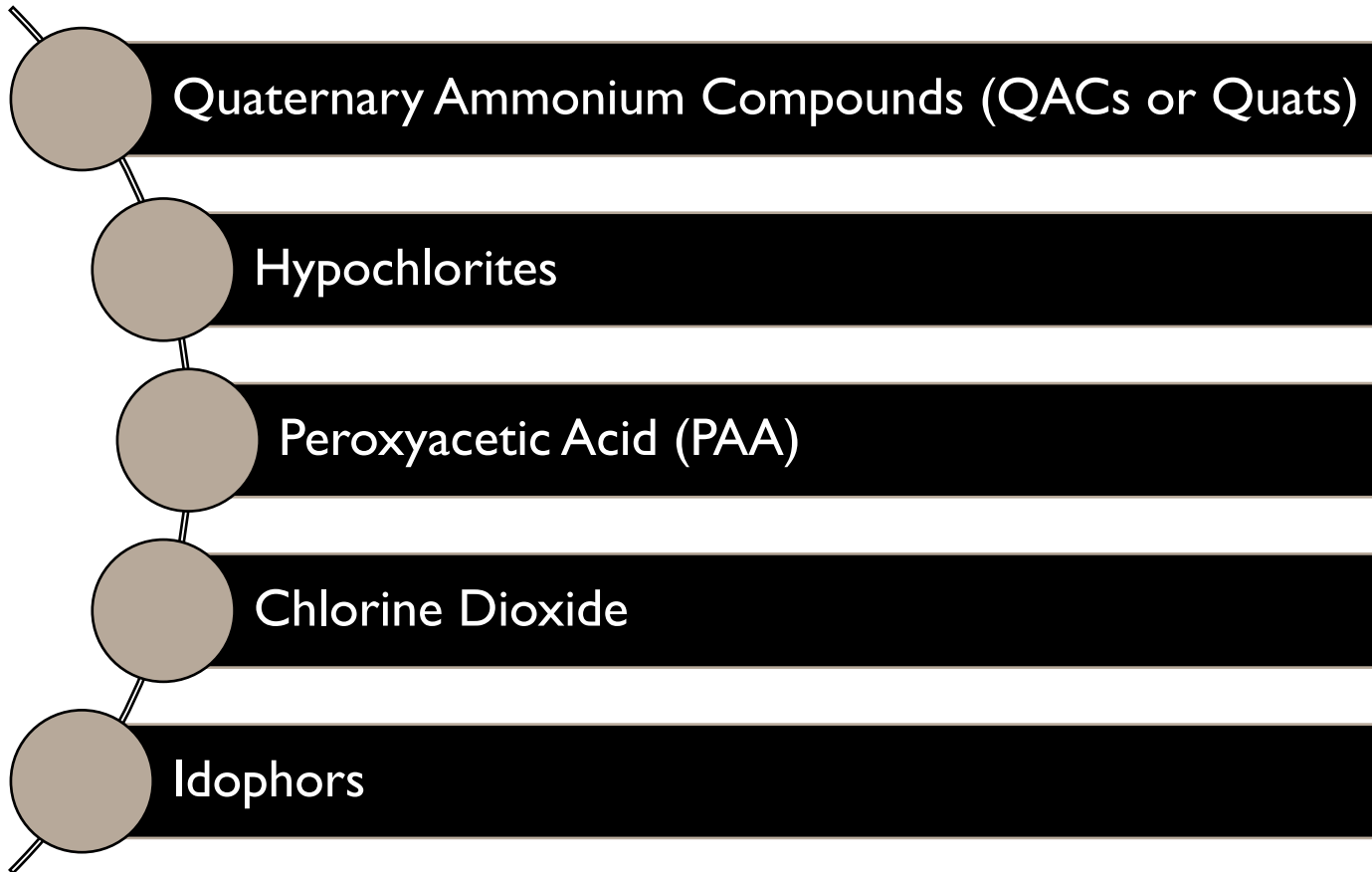
Resistance to several antimicrobials having unrelated targets or modes of action.

Often sequential linking of separate genes conferring resistance to different antibiotics, often on plasmids or integrons, and transferred together.

Cross-protection

Adaptation to one stress is associated with increased resistance to another, unrelated stress.

Common Sanitizers and Disinfectants in the Food Industry



No scientific evidence that sanitizers are ineffective *if* used according to the label and manufacturer recommendations!

But are they always used that way?

What is being reported?

Microbial species	Antimicrobial used	Resistance or reduced susceptibility developed...	Experimental approach	Reference
<i>Escherichia coli</i>	Triclosan	Levofloxacin, amoxicillin, tetracycline and chloramphenicol	Cells were exposed for 30 days to triclosan at a concentration of 0.2 mg/L.	Lu et al., 2018. Environ. Int. 118:257.
<i>Pseudomonas aeruginosa</i>	Benzalkonium chloride (BAC)	Polymyxin B, tetracycline, ciprofloxacin	Adaptive selective experiments were carried out for <i>P. aeruginosa</i> strains in the presence of BAC for more than 300 generations.	Kim et al., 2018. AEM. 84:e01201.
<i>Salmonella</i> Typhimurium	A mixture of aldehydes and QAC; a QAC; an oxidative compound; a halogenated tertiary amine compound	Nalidixic acid, ciprofloxacin, chloramphenicol, tetracycline	Bacterial cultures were repeatedly sub-cultured over 4 days (8 subcultures) in each biocide.	Webber et al., 2015. J. Antimicrob. Chemother. 70:2241.
<i>L. monocytogenes</i>	Ciprofloxacin (CIP); BAC	CIP-adapted or BAC-adapted strains with reduced susceptibility to gentamicin (GEN) and BAC, CIP, ethidium bromide, TPP	Bacterial cultures were repeatedly sub-cultured at 2 µg/ml CIP (2 subcultures) or 10 µg/ml BAC	Rakic-Martinez et al., 2011. AEM. 77:8714.
<i>L. monocytogenes</i>	Ciprofloxacin	CIP-adapted strains with reduced susceptibility to BAC & GEN (but only some strains)	Bacterial cultures were repeatedly sub-cultured to high CIP concentrations (30 to 240 µg/ml)	Kovacevic et al., 2013. Food Microbiol. 34:319.

What do we know about resistance/ tolerance/ susceptibility to QACs?

- “Resistance” is typically low-level
 - **Reduced susceptibility** or **increased tolerance** rather than resistance
 - Does not lead to resistance at concentrations recommended for use in the food industry
- Mechanisms
 - Reductions in cell permeability
 - Efflux pumps
 - *L. monocytogenes*: *bcrABC*, *emrE*, *emrC*, *qacC*, *qacH*

*QAC/QUAT, quaternary ammonium compounds

Elhanafi et al., 2010. AEM. 76:8231.

Muller et al., 2013. PLOS ONE. 8:e76835.

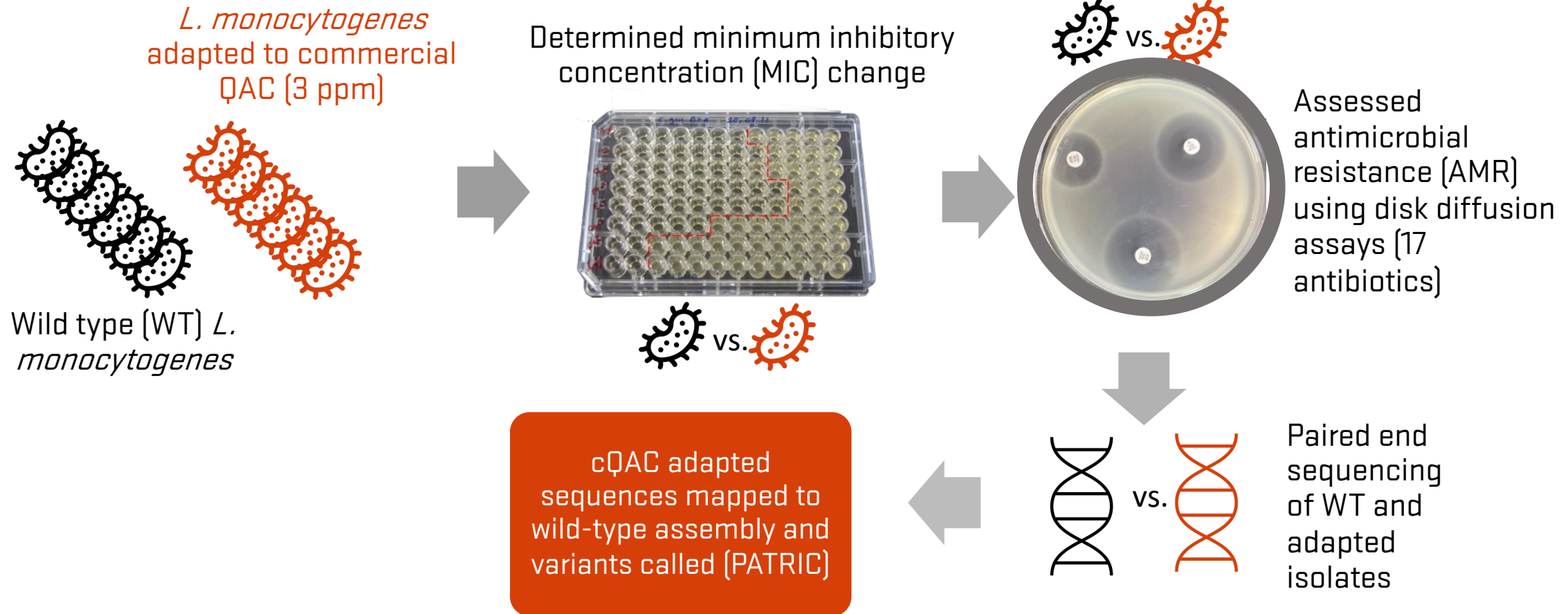
Kovacevic et al., 2016. AEM. 82:939.



Investigation of cross-resistance development between commercial sanitizers and antibiotics in *Listeria monocytogenes* isolated from food processing environments



Determining the potential for cross-resistance



Bland et al., 2022. *Frontiers in Microbiology* 2022, 12:782920.

<https://doi.org/10.3389/fmicb.2021.782920>



L. monocytogenes adapted to 3 ppm cQAC had decreased susceptibility to 7/17 antibiotics

- Genome wide analysis identified mutations in *fepR* regulator of FepA (multidrug efflux pump) across all adapted isolates tested.

Figure 5. Antibiotic susceptibility of wild-type (WT) and cQAC adapted (qAD) *Listeria monocytogenes* strains (n=6) to 17 antibiotics. Values reported represent zone diameters measured in mm. For adapted isolates, the median of 2-3 independent replicates is reported.

Antibiotic	WRLP354		WRLP380		WRLP394		WRLP483		WRLP530		WRLP533	
	WT	qAD	WT	qAD	WT	qAD	WT	qAD	WT	qAD	WT	qAD
	Isolate											
Amikacin	24	24	24	23	23	23	23	23	25	25	24	24
Ampicillin	27	25	27	24	27	24	26	22	28	23	30	24
Cefoxitin	10	9	9	10	9	10	10	11	9	10	10	10
Chloramphenicol	18	19	16	18	18	18	20	19	21	19	21	18
Ciprofloxacin	17	9	17	13	18	11	20	12	20	11	20	10
Clindamycin	10	14	15	14	13	13	13	13	14	13	14	13
Cotrimoxazole	29	28	29	24	27	24	25	25	31	30	31	31
Erythromycin	27	26	23	23	25	23	23	23	26	26	23	29
Gentamicin	22	19	18	18	20	17	20	20	22	17	22	18
Imipenem	27	34	29	32	28	32	29	30	32	30	34	30
Kanamycin	22	18	18	19	17	15	20	17	20	17	22	17
Novobiocin	23	15	18	14	19	17	17	15	23	15	23	18
Penicillin G	25	15	18	14	20	14	18	15	22	10	22	14
Rifampicin	25	27	25	25	24	26	24	25	27	27	26	28
Streptomycin	18	13	14	13	12	14	14	14	15	14	15	14
Tetracycline	30	22	24	22	23	24	23	21	27	22	23	22
Vancomycin	15	19	15	19	15	23	16	19	19	19	18	18

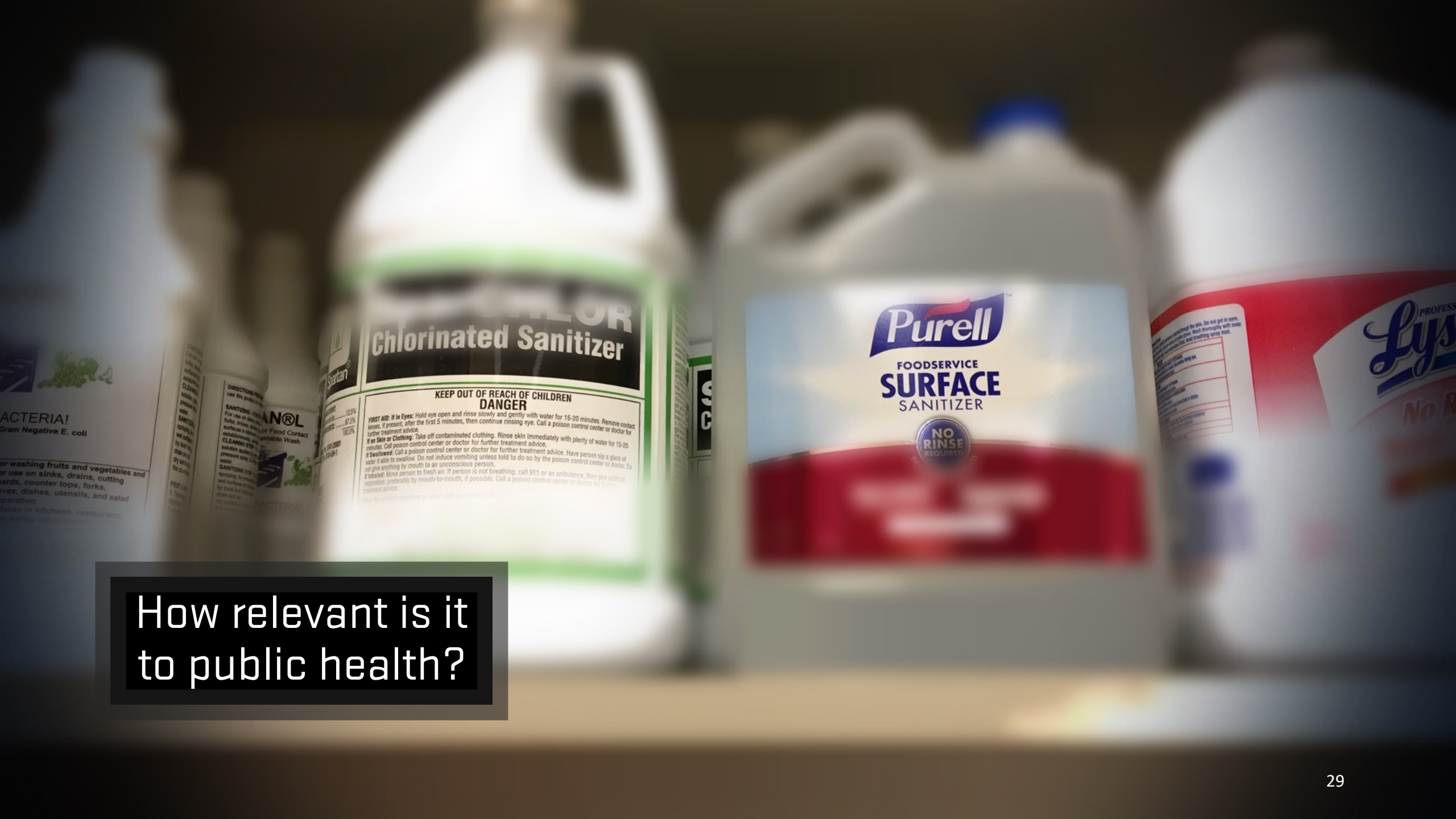


Data highlights

- There is a potential for cross-resistance development between cQAC and antibiotics of different classes.
- Mutations in the *fepR* regulator gene of the *fepA* multidrug efflux pump are contributing to cross-resistance to ciprofloxacin in *L. monocytogenes*.

Bland et al., 2022. *Frontiers in Microbiology* 2022, 12:782920.
<https://doi.org/10.3389/fmicb.2021.782920>





How relevant is it to public health?

AMR in the Food Industry – Is It Really Related to Sanitation?

“A review of recent literature reveals the lack of connection between resistance to antibiotics and biocides, **since real-world conditions are not consistently mimicked** and there is a **misunderstanding of terms**. The most common method used for this type of research is the MIC method, which has been criticized by experts in the field as **misrepresenting actual use conditions**. Non-substantiated conclusions have been drawn by researchers against standard sanitation **protocols that do not include effective cleaning followed by use of sanitizers** under required conditions and concentrations”.

Ruth Petran et al., 2018. IAFP. <https://iafp.confex.com/iafp/2018/meetingapp.cgi/Paper/17921>.



In our study....

We were not able to adapt isolates to high levels of commercial QAC.

While the potential for cross-resistance between a quaternary ammonium compound (QAC)-based sanitizer and select antibiotics exists, there has been no cross-resistance between antibiotics typically used to treat listeriosis (e.g., amikacin, gentamicin) and QAC, providing confidence in the continued use of these antibiotics as listeriosis treatment options.

Bland et al., 2022. *Frontiers in Microbiology* 2022, 12:782920.
<https://doi.org/10.3389/fmicb.2021.782920>



Antimicrobial resistance vs. tolerance in foodborne pathogen *L. monocytogenes*

“Research available to date fails to demonstrate ‘resistance’ of *L. monocytogenes* to recommended sanitizer treatments as prescribed by the label. As such, **sanitizer tolerance** would be a more accurate description of *L. monocytogenes* response to low sanitizer concentrations (i.e., sub-MRC). Conservative use of word ‘resistance’ will reduce confusion and allow for concise messaging as sanitizer research findings are communicated to industry and regulators.”



Summary

- Misuse of terms and non-realistic sanitizer application conditions in research potentially leading to reports of exaggerated “resistant” phenotypes
- No evidence that foodborne pathogens are becoming resistant to sanitation conditions recommended by sanitizer manufacturers
- Selective pressures occurring in food processing (e.g., sub-lethal sanitizer exposures, biofilms) can lead to sub-populations of environmental pathogens with tolerant phenotypes to sanitizers and other antimicrobials
 - Potential contributing factor to survival of microorganism with AMR markers
- Co-selection reported for foodborne pathogens but still lots of unknowns about mechanisms of resistance/tolerance, what triggers those events, and effects on public health
 - There is a potential for cross-resistance development between QACs and antibiotics of different classes – more research needed to understand this better

Bottom line:

Proper hygiene and food processing measures lower the risk of human exposure to antibiotic resistant bacteria originating from animals and external environments via food products.

I am happy to take questions

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